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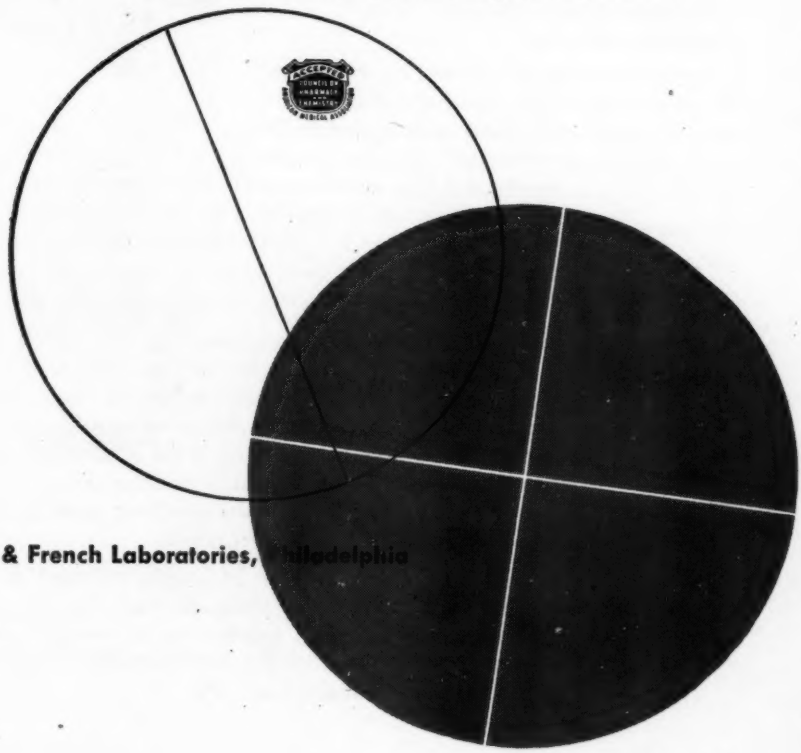

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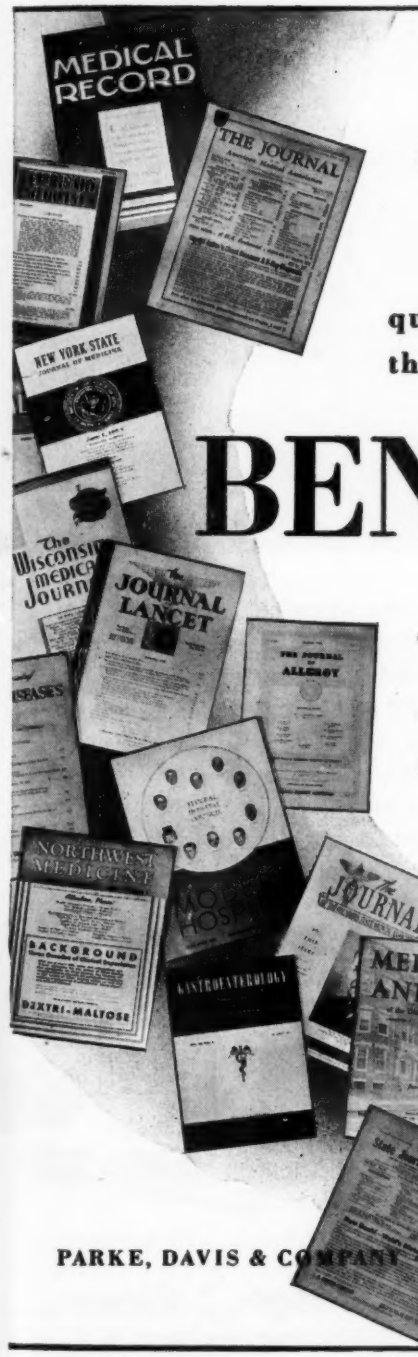

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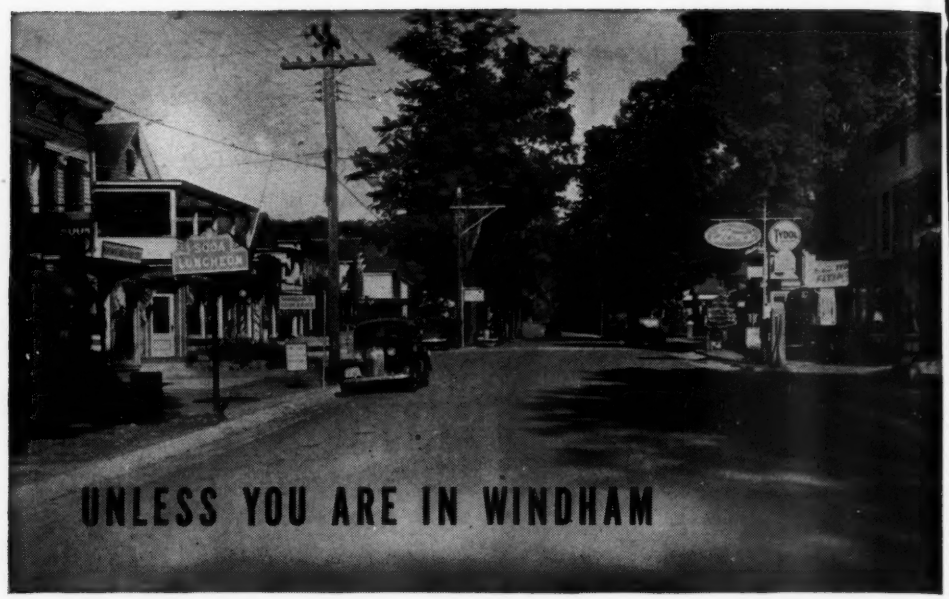

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E D I T O R I A L

THE A. PH. A. WAR MEMORIAL

THE conference and the dedication ceremony on the occasion of the presentation of the War Memorial to the American Pharmaceutical Association was an outstanding event in the annals of American pharmacy. The memorial itself was in the form of a flagstaff, the base of which depicts the activities of those pharmacists who have throughout our country's history served in the armed forces.

A number of points of outstanding significance relating to this meeting in Washington should not be overlooked by all those interested in the profession of pharmacy and its progress. First, credit is due to the donor of the memorial, Dr. H. A. B. Dunning, who once again has demonstrated both his generosity and his love for pharmacy. This he has done many times before and much of the progressive effort of the A. Ph. A. over the years has been financed and underwritten by this same generous spirit. The War Memorial itself is a fitting tribute to the service rendered by pharmacists in our country's wars. The flag that now flies proudly from this flagstaff on Constitution Avenue is a constant reminder that we did not and will not forget the labor and sacrifice of those who served their profession and their country in times of national emergency.

By far, however, the most significant and impressive fact about this occasion was the evidence of the new respect and esteem with which pharmacy is now held in many places where formerly it was given scant attention. This new attitude on the part of such key figures as the Surgeons General of the Army, the Navy and the Public Health Service is due to a number of influences. Lest there be some misunderstanding it should be pointed out that this change is not due solely to a less obstinate and unreconstructed viewpoint on the part of these important individuals. That their opinion of pharmacy has changed cannot be denied, but the reasons for this change are numerous. First, pharmacy today is doing a better all-around job than ever before in its history and the whole of civilian and military health is

more dependent on us than ever before. This fact was amply demonstrated in World War II and it has been recognozed fully by all the health professions and all agencies both private and public that make health their objective.

Last, but not least, pharmacy today is enjoying the fruits of good leadership and teamwork, an accomplishment that not too long ago was sorely needed. It is obvious that no one man deserves all the credit, but few can deny that the untiring efforts of the A. Ph. A. Secretary in directing and coordinating our efforts and contacts have been a major influence. His possession of the facts and their forceful presentation has been a bulwark in our efforts to strengthen pharmacy's position. The coordination with and the cooperation of the N. A. R. D. and its officers has been most beneficial as has that with the many other key organizations of pharmacy.

Pharmacists who served in the last war can look with satisfaction not only to the war memorial built in their honor, but with even more satisfaction to the fact that while they served their country, their recognition was strengthened immeasurably. That the war memorial and the center of the driving force should have the same address is appropriate indeed. That we shall go forward to even greater accomplishments seems a safe prediction providing we maintain that spirit of cooperative effort by which progress is made.

L. F. Tice.

A "SEMI-MICRO" METHOD FOR TESTING QUATERNARY AMMONIUM DISINFECTANTS

By Emil G. Klarmann and Eleanore S. Wright *

Introductory Note

OVER a period of several years it has become a matter of common experience that quaternary ammonium compounds do not yield uniform or consistent results when subjected to testing by the so-called F. D. A. "phenol coefficient" method (1). This inconsistency is evident not only in the case of different investigators testing a given quaternary ammonium disinfectant, but often too, in the case of the same investigator attempting to check his own findings. Although several different factors have been made responsible for this variability (2), we reported some time ago that the following two factors in carrying out the F. D. A. test are probably involved: firstly, the creation of a condition in the "medication" mixture (of diluted cationic disinfectant plus bacteria) which prevents the transfer of a truly representative bacterial sample into the subculture, and secondly, the failure to take due account of, and to suppress the characteristic and marked bacteriostatic action of the cationic compounds in the transfer tube (3). With reference to the former of the two factors, we showed that it was possible to modify the original F. D. A. method in one of two ways so as to eliminate the dissimilarity between the sample and the whole; as to the latter factor, we recognized the efficacy of serum in suppressing the troublesome bacteriostasis by the transferred cationic compounds, and we demonstrated the effect of employing this expedient upon the quantitative expression of their disinfectant potency, viz., in terms of their minimum germicidal concentrations acting in ten minutes, or of the phenol coefficients calculated therefrom.

Our considerations of the various relevant matters were and are predicated upon the fact that the F. D. A. "phenol coefficient" method, as an "end-method," requires a 100% kill by the lowest germicidal concentration of the disinfectant under test, as well as by that of the phenol standard. In this connection, it may be mentioned parenthet-

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ically that, under established regulatory procedure, the strength of the use solution of a given disinfectant is not identical with its lowest germicidal concentration (with respect to *E. typhosa*), as determined under the conditions of the F. D. A. testing method; rather the use solution is of a much higher concentration (viz., 4 to 4-1/4 times as strong) which in its performance corresponds to a 5% solution of phenol (4). Reference to these matters is made here because of certain questions which have been raised variously as to the justification of the demand for a 100% killing efficiency in ascertaining the lowest germicidal concentrations of cationic disinfectants (5). It is not our intention to debate here the merits or demerits of this issue; suffice it to say that in certain instances, at least, the requirement of a complete germicidal effect appears to be justified, as, e. g., in the disinfection of surgical instruments. With regard to the borderline between bacteriostatic and bactericidal concentrations it is well known that this line is not at all definite, but that it depends largely upon the treatment of the bacteria after their exposure to the action of the disinfectant solution. Thus O. Rahn (6) remarked correctly that bacteria, which after disinfection are unable to grow in broth, may still be able to multiply, e. g., in a wound. The relevance of such considerations to the problem of evaluation of "skin disinfectants" may be gathered, e. g., from the papers by W. J. Nungester and A. H. Kempf (7), by R. W. Sarber (8), and by other investigators working with cationics in comparative tests with other skin disinfectants. And finally, it should not be forgotten that the F. D. A. "phenol coefficient" method was recognized by its authors to lead to incorrect results if applied in its original form to antibacterial agents to which it is not suited for one reason or another. At the time of its publication, quaternary ammonium compounds did not play the role which they are playing today; no doubt, this is why no mention of them occurs in the classical Circular No. 198 of the U. S. Department of Agriculture. But the same publication refers to other materials such as, e. g., the organic mercurials which, because of their characteristic bacteriostatic action, are not fit subjects for testing by the original F. D. A. "phenol coefficient" procedure. Similarly, disinfectants containing chlorine or oxidizing agents do not lend themselves to testing by this method since their antibacterial action is markedly affected by organic matter. Incidentally, both considerations, viz., that of bacteriostasis and that of

the effect of organic matter, are known to apply to quaternary ammonium disinfectants (9). Thus it is only logical to postulate that any comparisons of the latter class with other classes of disinfectants are likely to be erroneous if based solely upon the results of the original F. D. A. "phenol coefficient" tests performed with members of the respective classes.[1]

"Semi-micro" Method Using Bacto-Oxgall as Suppressor of Bacteriostasis

In continuing our attempts at a narrower delineation of the disinfectant efficacy of quaternary ammonium compounds, we extended our search for more effective suppressors of their bacteriostatic action in subcultures. As indicated above, serum was found to be quite effective for this purpose, but its use involves certain inconveniences which are particularly evident in routine tests. The need for suppression of bacteriostasis by quaternary ammonium compounds was realized also by other investigators, some of whom suggested certain means for achieving this end. Reference is made here to the pertinent experiments with "Letheen" medium by A. Quisno, I. W. Gibby and M. J. Foter (11) because of the recognition which this work received in connection with the extensive collaborative studies participated in by eleven laboratories, under the leadership of L. S. Stuart (12). A somewhat similar medium was devised by E. H. Armbruster and G. M. Ridenour (13) who also called attention to the intense bacteriostatic action of the quaternary ammonium salts and its suppression by a special neutralizing formula.

In the present work the basic testing procedure was our "semi-micro" technic which, in our opinion, embodies a sound rationale with respect to quaternary ammonium disinfectants, for the reasons given in detail elsewhere (3). This technic calls for subculturing the entire medication mixture which, however, measures only one-tenth

[1] For the sake of completeness, mention should be made here of the work of L. H. Flett, R. C. Haring, A. F. Guiteras, and R. L. Shapiro (10), who showed that no absolute value should be ascribed to the germicidal concentrations of the phenol standard of the F. D. A. testing method. It was found that the sorption of phenol by the transferred test-organisms could be made to regress in such a fashion, as to shift the characteristic ten minute germicidal dilutions with respect to *E. typhosa* from 1:80 to 1:65, and with respect to *S. aureus* from 1:65 to 1:55 (or 1:50). This effect was produced by charcoal or ferric chloride operating as "inactivating" agents upon the bacteriostatic action of phenol. Its order of magnitude is around 20%, and is therefore very materially lower than that of the effect of suppressing the bacteriostasis of quaternary ammonium compounds referred to hereafter.

the volume of the corresponding mixture prepared under the conditions of the regular F. D. A. "phenol coefficient" method. Our latest refinement of the "semi-micro" method, aiming at an effective impairment of bacteriostasis (following the time interval of action of the cationic disinfectant upon the test organism), consists in the use of Bacto-Oxgall in the nutrient broth with which the medication mixture is diluted at the end of the test period. The preparation of the media and the operation of the "semi-micro" technic are described in the *Technical Part.* It should be mentioned here that whereas 1% of Bacto-Oxgall was found to be sufficient for our purpose in the case of *E. typhosa,* 5% is required in that of *S. aureus.* The superiority of Bacto-Oxgall as suppressor of bacteriostasis over several other relevant substances is illustrated by the following Table I, giving the minimum bacteriostatic concentrations of the Quaternary Ammonium Compound A under the conditions stated. The test was carried out by inoculating 20 cc. of broth containing a dilution of the Quaternary Ammonium Compound A with one 4 mm. loopful of the broth culture prepared in agreement with the F. D. A. method.

TABLE I

MINIMUM BACTERIOSTATIC CONCENTRATIONS OF QUATERNARY AMMONIUM COMPOUND A (100% ACTIVE INGREDIENT) IN DIFFERENT NUTRIENT BROTHS

F. D. A. Broth	F. D. A. Broth + 10% Serum	U. S. D. A "Letheen" Broth	Bacto-Oxgall Broth
	(A) *E. typhosa*		
1:60000— 1:80000+	1:10000— 1:20000+	1:8000— 1:10000+	1:200— 1:400+
	(B) *S. aureus*		
1:1000000— 1:2000000+	1:100000— 1:200000+	1:80000— 1:100000+	1:1000— 1:2000+

This table illustrates the very marked inhibitory action of a cationic compound, and considerably more so upon *S. aureus* than on *E. typhosa.*

Similar results were obtained in the case of other cationic disinfectants with respect to a very wide divergence between the minimum inhibitory concentrations in F. D. A. broth and in Bacto-Oxgall broth, respectively. The following Table II indicates the findings obtained with the several products studied. In these tests

20 cc. of regular F. D. A. broth, of 1% Bacto-Oxgall broth (for *E. typhosa*) and of 5% Bacto-Oxgall broth (for *S. aureus*) respectively, containing the various disinfectant dilutions, was inoculated with a 4 mm. loop of a 24 hour F. D. A. broth culture; the results were read after 48 hours of incubation. The designation + stands for the lowest disinfectant concentration permitting readily discernible growth in regular F. D. A. broth, X indicates the same in 1% Bacto-Oxgall broth (used with *E. typhosa*), and in 5% Bacto-Oxgall broth (used with *S. aureus*).

TABLE II

REDUCTION OF BACTERIOSTASIS BY THE USE OF BACTO-OXGALL BROTH

Quat. Amm. A 100%	Quat. Amm. B 100%	Quat. Amm. C 100%	Quat. Amm. D 100%	Dodecylamine 100%	Phenol
		(A) *E. typhosa*			
1:80000+	1:40000+	1:20000+	1:100000+	1:200000+	1:1000+
1:400×	1:200×	1:400×	1:200×	1:2000×	1:2000×
		(B) *S. aureus*			
1:2000000+	1:2000000+	1:2000000+	1:2000000+	1:200000+	1:600+
1:2000×	1:400×	1:4000×	1:2000×	1:600×	1:600×

Thus it appears from Table II that the bacteriostatic concentration of the Quaternary Ammonium Compound A is raised by the use of Bacto-Oxgall two-hundred times with respect to *E. typhosa* and one-thousand times with respect to *S. aureus;* similar findings were made with the other three cationic disinfectants. By contrast no such phenomenon is shown by phenol (which, for some unknown reason, appears to be slightly more bacteriostatic in the presence of Bacto-Oxgall than in its absence). Thus, Bacto-Oxgall appears to be somewhat of a specific in its capacity of neutralizing the bacteriostasis by quaternary ammonium compounds.

Germicidal Test Results

Having thus established the basis for the use of Bacto-Oxgall as a suppressor of the bacteriostatic action of quaternary ammonium disinfectants, one may now proceed to a consideration of the results obtained with the aid of the "semi-micro" technic as refined by the use of Bacto-Oxgall in subculturing the medication mixture. The following Table III states the results with *E. typhosa* as test organ-

ism obtained with the four quaternary ammonium disinfectants and phenol; the findings on the long-chain alkylamine, dodecylamine (brought into solution as the lactate), have been added because of our previous observations attesting to an essential similarity in the antibacterial action of long-chain alkylamines (or rather alkylammonium salts) and quaternary ammonium salts. Table IV gives the corresponding results obtained with *S. aureus*. Each disinfectant was tested six times. For reasons of time economy only ten minute contact periods were employed. The vertical columns of Tables III and IV describe the appearance of the subculture tubes following dilution of the 0.55 ml. of medication mixture (0.05 ml. of broth culture plus 0.5 ml. of disinfectant dilution) with 20 ml. of Bacto-Oxgall broth and incubation for 48 hours. Incidentally, the dilutions of the quaternary ammonium compounds are expressed in terms of 10% stock solutions rather than based upon their anhydrous contents. (This is deemed a fair procedure since many commercially available quaternary ammonium disinfectants are usually handled in concentrations closer to that of a 10% solution than to the anhydrous material.)

TABLE III

"Semi-Micro" Technic

E. typhosa-Bacto-Oxgall Broth

Diln.	Quat. Amm. A 10%	Diln.	Quat. Amm. B 10%	Diln.	Quat. Amm. C 10%
1:100	− − − − − −	1:1	+ + + + +	1:40	− −
1:200	+ − + − − +	1:2	+ +	1:60	− + − + − −
1:300	+ + − + + +	1:5	+ + +	1:80	− + − + + −
1:400	+ + + + + +	1:10	+ +	1:100	− + + + − −
1:500	+ + + + +	1:20	+	1:200	+ + + + +
1:600	+ +	1:30	+	1:300	+ + + +

Diln.	Quat. Amm. D 10%	Diln.	Dodecylamine 10%	Diln.	Phenol
1:1	+ + + + +	1:80	− − −	1:80	− − − − − −
1:2	+ + +	1:100	+ + + + − −	1:90	− + + + − +
1:5	+ + +	1:150	+ − − +	1:100	+ + + + + +
1:10	+ + +	1:200	+ + + + + +		
1:20	+ +	1:300	+ +		
1:40	+ +	1:400	+ +		

TABLE IV

"Semi-Micro" Technic

S. aureus-Bacto-Oxgall Broth

Diln.	Quat. Amm. A 10%	Diln.	Quat. Amm. B 10%	Diln.	Quat. Amm. C 10%
1:60	—	1:20	— —	1:60	— — — —
1:80	+ + —	1:40	+ — — — — —	1:80	+ + — — — —
1:100	— — + + — +	1:60	— — — — + +	1:100	+ — — — — —
1:200	— + + + — +	1:80	+ + + + + —	1:200	— + + + — +
1:300	+ + + + + +	1:100	+ — + — + +	1:300	+ + + + — +
1:400	+ + + + +	1:150	— + +	1:400	+ + +
1:500	+ + +	1:200	+ + + +		

Diln.	Quat. Amm. D 10%	Diln.	Dodecylamine 10%	Diln.	Phenol
1:20	— — — — — —	1:5	— —	1:50	— — — — — —
1:40	— — + — — +	1:10	+ + —	1:60	+ + — — + —
1:60	— + + + — —	1:20	+ — — — —	1:70	+ + + + + +
1:80	+ — — + + +	1:40	+ + + + —	1:80	+ + +
1:100	+ + + + + +	1:60	+ — + + + +		
1:200	+	1:80	+ + + +		
		1:100	+ + +		

With regard to *E. typhosa* as test organism (Table III) it appears that of the four quaternary ammonium salts tested, A (10%) and C (10%) disinfect completely, also upon dilution with water, where as B (10%) and D (10%) do not disinfect, in the proper sense of this term, even when used full strength (i. e., in 10% solutions). Beyond this, it is also evident that these compounds still do not give consistent results in all six tests although the divergence between the maxima and minima of the lowest germicidal concentrations has been narrowed down considerably. Thus the Quaternary Ammonium Compound A (10%) may be said to have an activity range betwen 1:100 and 1:300, C (10%) one between 1:40 and 1:150. Dodecylamine (10%) ranges from 1:80 to 1:150. A considerably narrower activity range is found in the case of phenol, viz., 1:80 to 1:90.

To illustrate the wide discrepancy between the F. D. A. phenol coefficients claimed for the cationic disinfectants in the literature and those obtained by the "semi-micro" method employing Bacto-Oxgall as suppressor of bacteriostasis, the following set of figures has been assembled in Table V. In this case, the average rather than the lowest effective concentrations obtained by the "semi-micro" method were used to calculate the phenol coefficients. Their F. D. A. phenol coefficients were taken or converted from published literature (to fit the 10% concentration of the stock solutions employed).

TABLE V

COMPARATIVE PHENOL COEFFICIENTS

Disinfectant	*E. typhosa*-Phenol Coeff. F. D. A. Method	*E. typhosa*-Phenol Coeff. Semi-Micro B. O. G.	*S. aureus*-Phenol Coeff. F. D. A. Method	*S. aureus*-Phenol Coeff. Semi-Micro B. O. G.
Quaternary Ammonium A (10%)	25	2.3	27.9	2.3
Quaternary Ammonium B (10%)	19	Not Germicidal	23	1.5
Quaternary Ammonium C (10%)	20	1.4	40	3.2
Quaternary Ammonium D (10%)	18	Not Germicidal	50	0.9
Dodecylamine (10%)	15	1.3	15	0.6
Phenol	1	1	1	1

The above table indicates that by using a testing method designed to take into account the peculiarities of this class of disinfectants, one obtains an idea of their germicidal performance which is different from that conveyed by reports in the literature and derived from tests

performed by the original F. D. A. "phenol coefficient" technic. Together with the information published previously, the present data support the contention that (a) the F. D. A. "phenol coefficient" method is not applicable to the evaluation of quaternary ammonium compounds, and (b) that the "phenol coefficients" variously claimed for these products cannot be true because of significant methodological errors inherent in the testing technic by which they have been obtained.

Comment

However, nothing that has been reported in this paper should be construed as either alleging or implying that quaternary ammonium compounds are ineffective or unreliable. On the contrary, it is readily conceded that these antibacterial agents have their proper place in the disinfectant picture, and that they are capable of performing a multitude of useful tasks. It is known that there exist many circumstances in which a 100% germicidal effect is not necessary, but where a very substantial reduction of the bacterial count is entirely adequate; this is probably true, e. g., of the sanitization of eating and drinking utensils, of food handling equipment, of milking appliances, etc., etc. Under such conditions, solutions of quaternary ammonium compounds of the correct strength and properly applied have been found to be entirely serviceable. While some problems still remain to be solved in certain phases of sanitization work, e. g., as pointed out by E. C. McCulloch (14), there is no doubt that intelligent application of these cationic disinfectants will achieve its purpose (15). By the same token, failure to take their peculiarities into account may involve certain risks under those conditions where a *full* germicidal effect is aimed at, without, however, being actually produced. With specific reference to the F. D. A. "phenol coefficient" test, it is held that this test is not properly applicable to the evaluation of quaternary ammonium disinfectants, that the strength of the use dilutions derived from its results may not be sufficient to accomplish the germicidal effect expected of them, and that an incorrect idea is obtained as to the comparative performance of the cationic and of other classes of germicides respectively, if derived solely from the consideration of their F. D. A. "phenol coefficients."

Technical Part

I. *Preparation of Bacto-Oxgall Media*

Depending upon whether *E. typhosa* (Hopkins) or *S. aureus* (209) is to serve as test-organism, 1% or 5% respectively, of Bacto-Oxgall is used. The composition and preparation for use with *E. typhosa* is as follows: 10 gms. of Armour's peptone, 5 gms. of Armour's beef extract, 10 gms. of Bacto-Oxgall in 1000 cc. of distilled water. Boil and adjust pH to 7.4. Autoclave in bulk for one-half hour. Add 5 gms. of "Super-Cel" (10 gms. in the case of 5% Bacto-Oxgall) and filter while hot. Add 5 gms. of dextrose per liter, tube 20 cc. per tube and autoclave for one-half hour at 15 pounds pressure.

II. *Details of the "Semi-Micro" Technic*

Pipet 0.05 cc. of a 24 hour F. D. A. broth culture of the test organism onto the bottom of sterile 25x150 mm. test tubes. This first step in the procedure must be done with great care so that the pipet does not touch the walls of the test tube. The tubes are then placed in a water bath at 20°C. Then 0.5 cc. of diluted disinfectant, which has also been kept in a water bath at 20°C., is added to each tube and thoroughly mixed with the culture. Ten minutes after adding the disinfectant, 20 cc. of Bacto-Oxgall broth is poured into the tube using aseptic precautions. The Bacto-Oxgall broth has previously been tubed 20 cc. per tube so that no delay in pouring is encountered. All tubes are incubated for 48 hours at 37°C.

Summary

Two characteristic factors interfere with the use of the regular F. D. A. "phenol coefficient" method in the evaluation of quaternary ammonium compounds, viz. (a) creation of a condition in the "medication" mixture which prevents the transfer of a representative bacterial sample into the subculture, and (b) failure to provide for effective suppression of the marked bacteriostatic action of the cationic compounds in the transfer tubes.

A "semi-micro" method has been developed intended to take care of these factors. It permits retention of the fundamental principles underlying the F. D. A. "phenol coefficient" method and their

application in the testing of these compounds. Bacto-Oxgall was found to be a specific neutralizer of the bacteriostasis produced by them. The results obtained with the aid of the "semi-micro" testing method, as refined by the use of Bacto-Oxgall in subculture media, suggest that the quaternary ammonium compounds are not entitled to the "phenol coefficient" figures obtained with the original F. D. A. method; conversely, the latter method does not appear to be directly applicable to the testing of quaternary ammonium compounds.

No such discrepancy between the results of the original F. D. A. and of the "semi-micro" methods, respectively, is observed in the case of phenol.

REFERENCES

(1) Klarmann, E. G., and Wright, E. S.: *Soap and Sanit. Chem. 21,* 113 (Jan. 1945).

(2) Brewer, C. M.: *J. Assn. Off. Agr. Chem. 27,* 554 (1944).

(3) Klarmann, E. G., and Wright, E. S.: *Soap and Sanit. Chem. 22,* 125 (Jan. 1946); *22,* 139 (Aug. 1946).

(4) Ruehle, G. L. A., and Brewer, C. M.: Circ. No. 198, U. S. Dept. of Agriculture.

(5) Mallmann, W. L., Kivela, E. W., and Turner G.: *Soap and Sanit. Chem. 22,* 130 (1946).

(6) Rahn, O.: *J. Am. Pharmac. Assn., Scientific Ed. 36,* 134 (1947).

(7) Nungester, W. J., and Kempf, A. H.: *J. Inf. Dis. 71,* 174 (1942).

(8) Sarber, R. W.: *J. Pharmacol. Exptl. Ther. 75,* 277 (1942).

(9) Klarmann, E. G., and Wright, E. S.: *Soap and Sanit. Chem. 20,* 103 (Feb. 1944).

(10) Flett, L. H., Haring, L. C., Guiteras, L. F., and Shapiro, R. L.: *J. Bacteriol. 50,* 591 (1945).

(11) Quisno, A., Gibby, I. W., and Foter, M. J.: *Am. J. Pharmacy 118,* 320 (1946).

(12) Stuart, L. S.: *Soap and Sanit. Chem. 23,* 135 (Sept. 1947).

(13) Armbruster, E. H., and Ridenour, G. M.: *Soap and Sanit. Chem. 23,* 119 (Aug. 1947).

(14) McCulloch, E. C.: *Science 105,* 480 (1947); *Amer. J. Pub. Health 38,* 493 (1948).

(15) Ridenour, G. M., and Armbruster, E. H.: *Amer. J. Pub. Health 38,* 504 (1948).

IODONIUM COMPOUNDS AND THEIR ANTIBACTERIAL ACTIVITY*

By Louis Gershenfeld and Bernard Witlin

THE iodonium compounds are members of a class of compounds in which iodine is present as an integral part of their structure as positive ions. They are strong bases and form stable salts when neutralized with acids. In this respect they behave as do the quaternary ammonium compounds and tertiary sulfonium compounds. They resemble the "onium" derivatives in that they are decomposed by heat. The temperature at which decomposition occurs varies with the electronegativity of the negative ion.

These positive valence iodine compounds are of great interest chemically due to the polyvalency of the iodine atom (the halogen atom exerts a valence greater than one). The investigations with this class of compounds for the past 50 years (especially between 1892 and 1912), have been primarily for the preparation and synthesis of the iodoniums from the standpoint of valence studies. Only occasional reference to this group is found in the literature within the past 30 years.

In 1885 Willgerodt (1) reported the first compound containing polyvalent iodine when he recorded the preparation of phenyliodoso chloride. In 1892, Meyer and Wachter (2) prepared the first iodoso compound, *o*-iodosobenzoic acid. Hartman and Meyer (3) in 1894 were the first to discover the existence of the iodonium compounds (a mixture of iodo and iodoso derivatives reacted with moist Ag_2O to form iodonium bases).

Many of the fundamental properties of the iodoso, iodoxy and iodonium groups have remained obscure or misapprehended since their discoveries by Willgerodt and Meyer. A monograph by Willgerodt (4) considers the work from 1885 up to 1914; Sandin (5) reviews the literature from 1914 to 1943. Recent contributions by Johnson (6), Lucas and coworkers (7), Mason and coworkers (8) and others have supplied data on the polyvalent iodine compounds and their interrelationship.

Gottlieb (9) referred to studies of the physiological reactions of the iodonium compounds on frogs and dogs by Hartman and

* This investigation was conducted at the Philadelphia College of Pharmacy and Science under a grant from the Iodine Educational Bureau's Industrial Fellowship at Mellon Institute.

Meyers. Paralysis of the muscles occurred with 0.02 Gm.-0.03 Gm. doses of diphenyliodonium chloride. This was of a peripheral nature and depended upon the paralysis of the motor nerve endings. The toxicology was analogous to metallic poisoning especially as found in lead or thallium poisoning. On account of the behavior on motor nerve endings, it was suggested that its action was similar to the ammonium bases.

Wertheim (10) states, "These compounds have no great importance but they display interesting properties." More recently the iodonium compounds have been studied to note whether they possess insecticidal properties. This presented the question as to whether they also may display bactericidal properties, so that as an aerosol or spray they may combine both insecticidal and bactericidal efficiencies.

While this article was in preparation, Freelander and French (11) reported on the toxicity and bacteriostatic activity of certain iodonium and sulfonium compounds. They reported high inhibitory powers against *Mycobacterium tuberculosis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Streptococcus pyogenes, Pseudomonas aeruginosa, Clostridium histolyticum* and *Monilia krusei* and attributed the activity of the iodonium compounds, in some cases, to their interaction with certain mercapto groups essential to the metabolism of the microorganisms. None of the compounds showed any significant therapeutic activity in guinea pigs.

Experimental

(A) Six iodonium compounds * in powder form were employed:

Compound No.	Compound
IB1	Diphenyliodonium chloride
IB4	Bis-*p*-chlorophenyliodonium sulfate
IB7	Bis-*p*-bromophenyliodonium iodide
IB6	Bis-*p*-chlorophenyliodonium iodide
IB8	Bis-*p*-iodophenyliodonium iodide
IB5	Diphenyliodonium iodide

The powder preparations were tested for bacteriostatic activity using the F. D. A. agar plate technic (12) and employing *Staphylococcus aureus* No. 209 (12) as the test organism (see Table I).

* The compounds were prepared and their solubilities were determined by the Iodine Educational Bureau Project at Battelle Memorial Institute, Columbus, Ohio.

Findings:

TABLE I

Bacteriostatic Activity of Iodonium Compounds (Powder)* Against *Staphylococcus aureus* by the Agar Plate Technic.

Compound No.	Zone in mm.
IB1	15
IB4	11
IB7	15
IB6	11
IB8	11
IB5	11

All of the iodonium compounds tested by the F. D. A. agar plate technic against *Staphylococcus aureus* displayed bacteriostatic zones of inhibition.

(B) Saturated aqueous solutions of the iodonium compounds were prepared. Five cc. of each were tested for bactericidal efficiency against 0.5 cc. of *Staphylococcus aureus* No. 209 at 37°C. employing F. D. A. standard loops (12) for transplants into 10 cc. quantities of F. D. A. media (12). (See Table II.)

The solutions were tested for free iodine with starch test solution U. S. P. (13).

Findings:

TABLE II

Bactericidal Efficiency of Iodonium Compounds (Saturated Aqueous Solutions) Employing *Staphylococcus aureus* at 37°C.

Compound	Solubility Gm./Liter H_2O	Minutes 1	2	3	4	5	10	15	30	45	60
IB1	7.38	+	+	+	+	+	+	+	+	+	+
IB4	8.74	0	0	0	0	0	0	0	0	0	0
IB7	0.096	+	+	+	+	+	+	+	+	+	+
IB6	0.14	+	+	+	+	+	+	+	+	+	+
IB8	0.19	+	+	+	+	+	+	+	+	+	+
IB5	0.40	+	+	+	+	+	+	+	+	+	+
Phenol 1:85		+	+	+	+	+	0	0	0	0	0

0 = No growth after 48 hours.
+ = Growth after 48 hours.

* The compounds were prepared and their solubilities were determined by the Iodine Educational Bureau Project at Battelle Memorial Institute, Columbus, Ohio.

Compound IB4, Bis-*p*-chlorophenyliodonium sulfate, was the only iodonium compound under test which displayed bactericidal efficiency against *Staphylococcus aureus* at 37°C. in saturated aqueous solution. All solutions tested were negative for free iodine.

(C) Dilutions were made of the saturated aqueous solutions of Bis-*p*-chlorophenyliodonium sulfate with sterile distilled water and tested for bactericidal efficiency against *Staphylococcus aureus* at 37°C. (See Table III.)

Findings:

TABLE III

Bactericidal Efficiency of Bis-*p*-chloropenyliodonium Sulfate (Saturated Aqueous Solution) Employing *Staphylococcus aureus* at 37°C.

	Minutes									
Aqueous Dilution IB4	1	2	3	4	5	10	15	30	45	60
Sat. aqueous sol.	0	0	0	0	0	0	0	0	0	0
1:2	0	0	0	0	0	0	0	0	0	0
1:4	0	0	0	0	0	0	0	0	0	0
1:6	+	+	+	+	+	+	+	+	+	+
1:8	+	+	+	+	+	+	+	+	+	+
Phenol 1:85	+	+	+	+	+	0	0	0	0	0

0 = No growth after 48 hours.
+ = Growth after 48 hours.

All dilutions gave negative results for free iodine.

(D) In order to ascertain whether the iodine in the iodonium compound would be inactivated by the addition of sodium thiosulfate, a series of tests were repeated as in "C" except that a sterile aqueous 10 per cent solution of sodium thiosulfate was employed for making the dilutions instead of sterile distilled water. A control of the 10 per cent sodium thiosulfate was run simultaneously.

Findings:

The addition of sodium thiosulfate to the bis-*p*-chlorophenyliodonium sulfate did not affect the bactericidal efficiency of the compound. Sodium thiosulfate in ten per cent solution did not possess bactericidal efficiency.

(E) Saturated solutions of the iodonium compounds were prepared in ethyl alcohol (95 per cent) and tested for bactericidal efficiency against *Staphylococcus aureus* at 37°C. F. D. A. agar cup plates (12) were also prepared (see Table IV).

Findings:

TABLE IV

Bactericidal Efficiency of Iodonium Compounds (Saturated Solution in Ethyl Alcohol [95 per cent]) Against *Staphylococcus aureus* at 37°C.

Compound	Dilution Sterile Dist. Water	Minutes 1	5	10	15	30	45	60	90	Agar Cup Zone in mm.	Free Iodine
	S	0	0	0	0	0	0	0	0	13	—
IB1	1:2	+	+	+	+	+	+	+	+		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	14	—
IB4	1:2	0	0	0	0	0	0	0	0		
	1:4	0	0	0	0	0	0	0	0		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	10	—
IB7	1:2	0	0	0	0	0	0	0	0		
	1:4	0	0	0	0	0	0	0	0		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	10	—
IB6	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	5	—
IB8	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	13	—
IB5	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
Control	S	+	+	+	+	+	+	+	+		
Alcohol 95%	1:2	+	+	+	+	+	+	0	0	3	
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
Phenol	1:85	+	+	0	0	0	0	0	0	0	

0 = No growth after 48 hours.
+ = Growth after 48 hours.
S = Saturated solution.

(F) Saturated solutions of iodonium compounds were prepared in a mixture of acetone, 10 parts by volume with 90 parts ethyl alcohol (95 per cent) by volume and tested for bactericidal efficiency against *Staphylococcus aureus* (as in E) (see Table V).

Findings:

TABLE V

Bactericidal Efficiencies of Iodonium Compounds (Saturated Solution in Acetone-Alcohol Against *Staphylococcus aureus* at 37°C.

Compound	Dilution Sterile Dist. Water	Minutes 1	5	10	15	30	45	60	90	Agar Cup Zone in mm.	Free Iodine
	S	0	0	0	0	0	0	0	0	15	—
IB1	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	20	—
IB4	1:2	0	0	0	0	0	0	0	0		
	1:4	0	0	0	0	0	0	0	0		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	15	—
IB7	1:2	0	0	0	0	0	0	0	0		
	1:4	0	0	0	0	0	0	0	0		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	10	—
IB8	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
	S	0	0	0	0	0	0	0	0	13	—
IB5	1:2	0	0	0	0	0	0	0	0		
	1:4	+	+	+	+	+	+	+	+		
	1:8	+	+	+	+	+	+	+	+		
Control	S	+	+	+	0	0	0	0	0		
10% acetone	1:2	+	+	+	+	0	0	0	0		
in alcohol	1:4	+	+	+	+	+	+	+	+	3	
	1:8	+	+	+	+	+	+	+	+		
Phenol	1:85	+	+	0	0	0	0	0	0	0	

0 = No growth after 48 hours.
+ = Growth after 48 hours.

(G) A saturated acetone-alcohol solution (as in F) of bis-*p*-chlorophenyliodonium sulfate was prepared. The solubility of the compound in the solvent mixture was determined. Bactericidal efficiency tests were conducted at 37°C. against *Staphylococcus aureus, Eberthella typhosa, Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, Proteus vulgaris* and *Bacillus subtilis.*

One cc. quantities from each test tube showing no growth after 48 hours were subcultured into another tube or broth (10 cc.) to test for bacteriostasis. (See Table VI.)

Findings:

Bis-*p*-chlorophenyliodonium sulfate was soluble in approximately one per cent concentrations in the acetone-alcohol mixture.

When quantities of 1 cc. and less were subcultured in 10 cc. of similar media to test for bacteriostasis, all transplants were negative (did not show growth).

A saturated solution of Bis-*p*-chlorophenyliodonium sulfate in acetone-alcohol when tested at 37°C. revealed bactericidal efficiency in one minute against: *Serratia marcescens* and *Eberthella typhosa* when diluted 1 to 19; *Escherichia coli* when diluted 1 to 13; *Proteus vulgaris* when diluted 1 to 18; *Staphylococcus aureus* when diluted 1 to 4 with sterile distilled water.

Bacillus subtilis was not killed by the saturated acetone-alcohol solution.

TABLE VI

Bactericidal Efficiency of Bis-*p*-chlorophenyliodonium Sulfate (Saturated Solution in Acetone-Alcohol) at 37° C.

	Serratia marcescens							*Eberthella typhosa*							*Pseudomonas aeruginosa*						
	Minutes							Minutes							Minutes						
Dilution	1	5	10	15	30	45	60	1	5	10	15	30	45	60	1	5	10	15	30	45	60
1:5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0
1:11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:20	+	+	+	+	+	0	0	+	+	+	+	+	0	0	+	+	+	+	+	+	+
1:30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

0 = No growth after 48 hours.
+ = Growth after 48 hours.

TABLE VI, cont'd.

Bactericidal Efficiency of Bis-*p*-chlorophenyliodonium Sulfate (Saturated Solution in Acetone-Alcohol) at 37° C.

	Escherichia coli							*Proteus vulgaris*							*Bacillus subtilis*						
	Minutes							Minutes							Minutes						
Dilution	1	5	10	15	30	45	60	1	5	10	15	30	45	60	1	5	10	15	30	45	60
1:5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:14	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:15	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:16	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:17	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:18	+	+	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	+	+	+
1:19	+	+	+	+	+	+	+	+	+	+	0	0	0	0	+	+	+	+	+	+	+
1:20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

0 = No growth after 48 hours.
+ = Growth after 48 hours.

(H) The tests as given under "G" were repeated employing sodium thiosulfate (10 per cent aqueous solution) as the diluent instead of sterile distilled water.

Findings: The addition of sodium thiosulfate did not affect the bactericidal efficiency of bis-*p*-chlorophenyliodonium sulfate.

(I) Bactericidal efficiency tests were performed on a 24-hour old culture of *Bacillus subtilis* and sporicidal efficiency tests were performed on a 4-day old culture of *Bacillus subtilis* growing in F. D A. broth at 37°C. These were conducted at 37°C. and 25°C. Subcultures of transplant tubes showing no growth after 48 hours were transferred to 10 cc. quantities of F. D. A. broth to test for bacteriostasis. (See Tables VII and VIII.)

TABLE VII

Bactericidal Efficiency of Bis-*p*-chlorophenyliodonium Sulfate (Saturated Solution in Acetone-Alcohol) on *Bacillus subtilis* (24-hour culture).

	Time in Hours									
Temperature	½	1	1½	2	2½	3	3½	4	4½	5
37° C.	+	+	+	+	+	+	+	0	0	0
25° C.	+	+	+	+	+	+	+	+	+	+

TABLE VIII

Sporicidal Efficiency of Bis-*p*-chlorophenyliodonium Sulfate (Saturated solution in Acetone-Alcohol) on *Bacillus subtilis* (4-day old sporulated culture).

	Time in Hours											
Temperature	½	1	1½	2	2½	3	3½	4	4½	5	6	7
37° C.	+	+	+	+	+	+	+	0	0	0	0	0
25° C.	+	+	+	+	+	+	+	+	+	+	+	+

TABLE VIII (cont'd.)

	Time in Hours															
Temperature	8	9	10	11	12	24	36	48	72	96	120	144	168	192	216	240
37° C.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25° C.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Findings:

Bis-*p*-chlorophenyliodonium sulfate in saturated alcohol-acetone solution was capable of killing *B. subtilis* spores in 4 hours at 37°C. This solution was devoid of effect in killing *B. subtilis* spores within 240 hours at 25°C.

Summary and Conclusions

The iodonium compounds are members of a class of compounds in which iodine is an integral part of their structure present as positive ions. They have only recently been studied from the standpoint of their practical value.

Antibacterial efficiency tests of several iodonium compounds were conducted with diphenyliodonium chloride, bis-*p*-chlorophenyliodonium sulfate, bis-*p*-bromophenyliodonium iodide, bis-*p*-chlorophenyliodonium iodide, bis-*p*-iodophenyliodonium iodide and diphenyliodonium iodide.

(1) These iodonium compounds (in powder form) displayed bacteriostatic activity when tested by the F. D. A. agar plate technic (inhibition zones from 11 to 15 mm.).

(2) Only bis-*p*-chlorophenyliodonium sulfate in saturated aqueous solution (even when diluted 1:4) displayed bactericidal efficiency against *Staphylococcus aureus* at 37°C. within 1 minute.

(3) The addition of sodium thiosulfate did not affect the bacteriostatic or bactericidal efficiencies of the iodonium compounds. Solutions did not reveal the presence of free iodine.

(4) Saturated solutions of the iodonium compounds in ethyl alcohol (95 per cent) displayed bactericidal efficiencies greater than alcohol itself, against *Staphyloccus aureus* at 37°C. within 1 minute.

(5) Saturated solutions of all the iodonium compounds in a solvent consisting of 10 per cent acetone by volume in alcohol (95 per cent) displayed bactericidal efficiency against *Staphylococcus aureus* at 37°C. within 1 minute.

(6) A saturated solution of bis-*p*-chlorophenyliodonium sulfate in acetone-alcohol solvent displayed bactericidal efficiency within 1 minute against *Eberthella typhosa, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli* and *Proteus vulgaris.*

(7) A saturated alcohol-acetone solution of bis-*p*-chlorophenyliodonium sulfate (even in dilution) was capable of killing *Bacillus subtilis* (24-hr. old culture) and *B. subtilis* spores (4-day old culture) within 4 hours at 37°C. but was devoid of killing effect within 240 hours at 25°C.

BIBLIOGRAPHY

(1) Willgerodt, C.: *J. Prakt. Chem. 33*:154 (1886).

(2) Meyer, V., and Wachter, W.: *Ber. 25*:2632 (1892).

(3) Hartmann, C., and Meyer, V.: *Ber. 26*:1727 (1893).

(4) Willgerodt, C.: "Die Organischen Verbindungen mit mehrwertigen Jod." F. Enke, Stuttgart (1914).

(5) Sandin, R. B.: *Chem. Rev. 32*:249 (1943).

(6) Johnson, J. R.: "Gilman's Organic Chemistry," Vol. II, p. 1612. John Wiley & Sons. New York (1938).

(7) Lucas, H. J.: *J. Am. Chem. Soc. 63*:2541 (1941).

(8) Mason, I.: *J. Chem. Soc.* p. 1699 (1938).

(9) Gottlieb, R.: *Ber. 27*:1599 (1894).

(10) Wertheim: "Textbook of Organic Chemistry" (2nd Ed.), p. 434. Blakiston Co., Philadelphia, Pa. (1945).

(11) Freedlander, B. L., and French, F.: *Proc. Soc. Exp. Biol. & Med. 63*:319, Article No. 15,587 (Nov., 1946).

(12) Ruehle, G. L. A., and Brewer, C. M.: "U. S. Food and Drug Administration Methods of Testing Antiseptics and Disinfectants." U. S. Department of Agriculture, Circular No. 198 (1931).

(13) *Pharmacopoeia of the United States* (Thirteenth Revision), p. 848 (1947).

THE BACTERIOSTATIC EFFICIENCY OF IODONIUM COMPOUNDS

(Toxicity studies of certain members of this group)*

By Louis Gershenfeld and Bernard Witlin

IODONIUM compounds, possessing iodine as positive ions in their structure, have previously been reported to display bactericidal activity (1) as well as bacteriostasis (2). The investigations by Freedlander and French (2) were concerned with the growth inhibiting qualities of the iodonium compounds under test. They incorporated aqueous solutions of the iodonium compound into broth or agar media. The latter were seeded with pyogenic strains of the test organisms: *Staphylococcus aureus, E. coli, P. vulgaris, Strep. pyogenes, Ps. pyocyaneus* and *Cl. histolyticum.* The dilutions capable of inhibiting their growth were noted. Several of the diphenyliodonium derivatives were also investigated by Freedlander and French (2) for their (*in vivo*) pharmacologic action on tuberculous guinea pigs. The pathology observed was evaluated numerically by the method of Sweany, Sher and Kloeck (3). The maximum gross involvements of the organs were given numerical ratings and an "Average Pathological Rating" was reported.

Since Freedlander and French's (2) bacteriostatic studies were performed incidental to the possible chemotherapeutic activities of the iodonium compounds and on a different compound than that previously reported by us, it was deemed advisable to study the bacteriostatic properties of the iodonium compounds upon which bactericidal efficiencies had been reported (1).

Saturated aqueous solutions of the following compounds were prepared in sterile distilled water: Diphenyliodonium chloride[1] (0.738%); Bis-*p*-chlorophenyliodonium sulfate[1] (0.874%); Bis-*p*-bromophenyliodonium iodide[1] (0.0096%); Bis-*p*-chlorophenyliodonium iodide[1] (0.014%); Bis-*p*-iodophenyliodonium iodide[1] (0.019%) and Diphenyliodonium iodide[1] (0.04%).

* This investigation was conducted at the Philadelphia College of Pharmacy and Science, under a grant from the Iodine Educational Bureaus Fellowship at Mellon Institute.

[1] The compounds were prepared and their solubilities were determined by the Iodine Educational Bureau Project at Battelle Memorial Institute, Columbus, Ohio.

Bacteriostatic Efficiency Tests

Bacteriostatic efficiency tests were conducted with the iodonium compounds, employing the following three techniques.

(a) Kolmer Bacteriostatic Technique (4):

One cc. of a 24-hour broth culture of the test organism was added to 99 cc. of sterile broth. After mixing well, 9 cc. of the mixture were aseptically pipetted into sterile tubes containing 1 cc. of the dilution of the compound under test. These mixtures were incubated for 48 hours at 37°C. and bacteriostasis was determined by the appearance of growth as compared with controls.

(b) Employing 2X broth, dilutions of the compound under test were made with distilled water so that the concentration desired would be present in amounts of 10 cc. One-tenth cc. of a 24-hour broth culture of the test organism was added to each tube. These tubes were incubated for 48 hours at 37°C. and the absence or presence of growth was noted.

(c) To parallel the F. D. A. phenol coefficient technique (5), 5 cc. of each dilution of the compound under test prepared with distilled water was mixed with 0.5 cc. of a 24-hour broth culture of the test organism. A standard F. D. A. 4 mm. loopful of each dilution-bacteria mixture was transferred to 10 cc. of sterile broth. These were incubated for 48 hours at 37°C. The absence or presence of growth was noted. The tubes showing no growth were tested for bacteriostasis by transferring 4 standard F. D. A. (4 mm.) loopfuls to tubes containing 10 cc. of sterile broth. These tubes were incubated at 37°C. for 48 hours.

Test Organisms

The test organisms employed were *Staphylococcus aureus, Serratia marcescens, Eberthella typhosa, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris,*[2] *Bacillus subtilis, Bacillus mesentericus,*[2] *Bacillus megatherium*[2] and *Streptococcus hemolyticus.* All tests were conducted in F. D. A. broth except when using the streptococcus, when brain-heart infusion broth (Difco) was employed.

Identical findings were noted in all three bacteriostatic techniques used in this study. The results obtained are recorded in the following table.

[2] Vegetative forms and spores were used separately.

MINIMUM CONCENTRATIONS OF IODONIUM IONS SHOWING BACTERIOSTASIS

Test Organism	Diphenyliodonium Chloride mg./cc.	Diphenyliodonium Iodide mg./cc.	Bis-*p*-chlorophenyliodonium Sulfate mg./cc.	Bis-*p*-chlorophenyliodonium Iodide mg./cc.	Bis-*p*-bromophenyliodonium Iodide mg./cc.	Bis-*p*-iodophenyliodonium Iodide mg./cc.
Staphylococcus aureus	.007	.013	.008	.001	.001	.0019
Serratia marcesens	.007	(a)	.8	(c)	.009	(e)
Eberthella typhosa	.7	(a)	(b)	(c)	(d)	(e)
Pseudomonas aeruginosa	.07	(a)	.8	(c)	.009	(e)
Escherichia coli	.07	(a)	.8	(c)	(d)	(e)
Proteus vulgaris	.007	(a)	.8	(c)	.009	(e)
B. subtilis (vegetative)	.007	(a)	.08	.001	.009	.019
B. subtilis (spores)	.007	(a)	.08	.001	.009	.019
B. mesentericus (vegetative)	.007	.04	.008	.014	.009	.019
B. mesentericus (spores)	.007	.04	.008	.014	.009	.019
B. megatherium (vegetative)	.007	.04	.008	.014	.009	.019
B. megatherium (spores)	.007	.04	.008	.014	.009	.019
Streptococcus hemolyticus PY8	.009	.04	.003	.014	.009	.019

(a) Greater than 0.4 mg./cc.
(b) Greater than 8.0 mg./cc.
(c) Greater than 0.1 mg./cc.
(d) Greater than 0.09 mg./cc.
(e) Greater than 0.19 mg./cc.

Toxicity Studies

Toxicity studies on diphenyliodonium chloride and bis-*p*-chlorophenyliodonium sulfate were conducted, employing 30 gram white mice (Wistar strain). Aqueous solutions of the above iodonium compounds were injected intraperitoneally into the test animals.

Findings

The toxic dose for both diphenyliodonium chloride and bis-*p*-chlorophenylidonium sulfate was 20 mg./kilogram of body weight.

All animals receiving these iodonium compounds showed an increased excitability shortly following injection, as evidenced by washing of the fore-paws. Their respiration rate increased considerably and a paralysis of the hind legs resulted. Those animals receiving sub-lethal doses recovered fully and no visible symptoms of drug action remained.

Summary and Conclusions

(1) Bacteriostatic efficiency tests were performed on the following iodonium compounds in aqueous solution: Diphenyliodonium chloride, bis-*p*-chlorophenyliodonium sulfate, bis-*p*-bromophenyliodonium iodide; bis-*p*-chlorophenyliodonium iodide and diphenyliodonium iodide. The bacteriostatic efficiencies of these iodonium compounds were tested against *Staphylococcus aureus, Serratia marcescens, Eberthella typhosa, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Bacillus subtilis, Bacillus mesentericus, Bacillus megatherium* and *Streptococcus hemolyticus.*

(2) The minimum bacteriostatic concentration of the iodonium compounds varied from 0.001 mg./cc. for bis-*p*-chlorophenyliodonium iodide (*Staphylococcus aureus*) to 0.8 mg./cc. for bis-*p*-chlorophenyliodonium sulfate (*Proteus vulgaris*). The bacteriostatic efficiency against some of the test organisms was greatest for bis-*p*-chlorophenyliodonium iodide, followed by bis-*p*-iodophenyliodonium iodide.

It is of interest to note that three of these iodonium compounds bis-*p*-chlorophenyliodonium iodide, bis-*p*-iodophenyliodonium iodide and diphenyliodonium iodide displayed bacteriostatic properties only against the gram positive test bacteria employed, including spore formers, but possessed no effect against the gram negative organisms

used in this study. Diphenyliodonium chloride was effective against all of the test bacteria (gram positive and gram negative, including spore formers). Bis-*p*-chlorophenyliodonium sulfate had no effect against *E. typhosa;* bis-*p*-bromophenyliodonium iodide had no effect against *E. typhosa* and *E. coli;* but both compounds displayed bacteriostatic properties against the other test organisms.

(3) In the case of the diphenyliodonium salts, the chloride has a much greater bacteriostatic efficiency than the iodide in all except possibly two cases, against *Staphylococcus aureus and Eberthella typhosa.* With the bis-*p*-chlorophenyliodonium salts the iodide is more effective than the sulfate in three of the eight cases in which a comparison is possible and about equally effective in the others. It is also to be noted that, in general, bis-*p*-chlorophenyliodonium iodide is about as effective as diphenyliodonium chloride. These observations suggest that bis-*p*-chlorophenyliodonium chloride should have better bacteriostatic properties than any of the compounds mentioned.

(4) In the over-all picture, considering all test bacteria, diphenyliodonium chloride appears as the most generally effective substance. It was bacteriostatic to all organisms tested, only one, *Eberthella typhosa,* requiring a concentration higher than the saturation concentration of the compound of minimum solubility, bis-*p*-bromophenyliodonium iodide. Furthermore, it showed bacteriostatic efficiency at lower concentrations than any of the other compounds tested in the case of six of the eleven organisms for which a reasonable comparison is possible.

Bis-*p*-bromophenyliodonium iodide appears as second in general effectiveness. It was ineffective against two organisms; in one case, *Eberthella typhosa,* this can be attributed to its low solubility, while there appears to be a possibility that it would have been effective against *Escherichia coli* in concentrations approaching saturation. Its effective concentration was lower than that of all other compounds in only two cases, but it was generally effective at the relatively low concentration of 0.009 mg./cc.

Bis-*p*-chlorophenyliodonium sulfate was bacteriostatic to one more organism than bis-*p*-bromophenyliodonium iodide but it required concentrations much higher than the saturation value of the other in four cases and almost as high in two cases.

Bis-*p*-chlorophenyliodonium iodide was most effective in only two cases, required 100% greater concentration than diphenyliodonium chloride and a 50% greater concentration than bis-*p*-bromophenyliodonium iodide in five cases. It was ineffective in five cases even though its solubility is 50% greater than that of the bromo compound.

Bis-*p*-iodophenyliodonium iodide required higher concentrations than all other compounds except diphenyliodonium iodide to be effective against five organisms, was fourth most effective against two, third most effective against one and was completely ineffective against five organisms even though its solubility is twice that of the bromo compound.

(5) It is of interest to note that when comparing the results of diphenyliodonium chloride and iodide with those of bis-*p*-chlorophenyliodonium sulfate and iodide, it appears that it is the anion which has the effect on the activity.

(6) White mice (Wistar strain) were given intraperitoneal injections of diphenyliodonium chloride and bis-*p*-chlorophenyliodonium sulfate. Animals receiving injections of these iodonium compounds revealed increased excitability, increased respiration and paralysis of the hind legs. The lethal dose was 20 mg./kilogram of body weight for both of the compounds tested.

BIBLIOGRAPHY

(1) Gershenfeld, L., and Witlin, B.: Abst. of Proceedings, 47th General Meet., Soc. Am. Bact. *33* (May, 1947).

(2) Freedlander, B., and French, F.: *Proc. Soc. Exp. Biol. & Med. 63*:319 (Nov., 1946).

(3) Sweany, H. C., Sher, B. C., and Kloeck, J. M.: *Am. Rev. Tuberc. 53*:254 (1946).

(4) Kolmer, J., and Boerner, F.: "Approved Laboratory Technic" (3rd Ed.). Appleton Century (N. Y., 1941).

(5) Ruehle, G. L. A., and Brewer, G. M.: "United States Food and Drug Administration Methods of Testing Antiseptics and Disinfectants." U. S. Dept. of Agriculture Circular No. 198 (1931).

EQUIPMENT FOR FIRST AID

By Leonard D. Frescoln, M. D.

IT is of prime importance in First Aid work to have at hand equipment with which to work, as well as the knowledge of how to use it. Indeed it is surprising to find how many people are trained as to what to do in emergencies and yet lack the equipment with which correctly to put into practice their training. It is true that even though one has on hand a pocket First Aid kit there may be need of improvising further in order to meet the particular problem. Also the worker should see to it that the contents of the kit are kept replenished from time to time.

In the case of a physician a special Medical Outfit is to be carried, and even more inclusive equipment is to be carried in the auto or ambulance.

In the home, and especially in the hospital, still more elaborate provision is to be had for the more complete handling of emergencies.

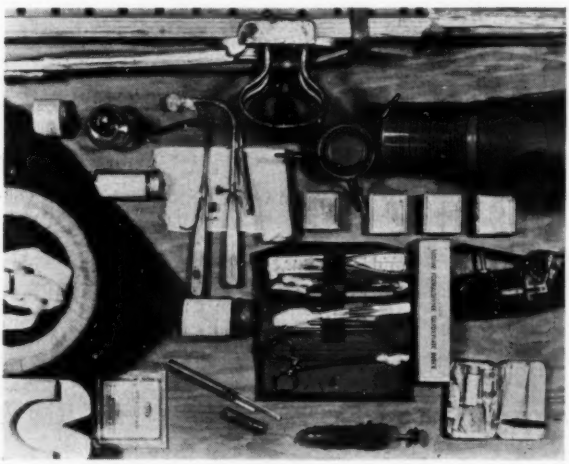
First, let us suggest a pocket kit for the one taking a First Aid Course. Get a small metal box, such as the one that used to be made for containing wire-nails, with lid opening to full view of the contents, or one of the "Band-aids" containers, one that will be carried habitually in the ordinary pocket or pocketbook. It should contain:

Plain Band-aids
Medicated (e. g. Mercurochrome) Band-aids
Vial or ampul of Aromatic Spirit of Ammonia
Good size pearl of castor oil for eye or ear application
Small tube of burn ointment
Small vial of oil of clove for toothache
Absorbent cotton
Small splinter forceps
Needle
Small container of sodium bicarbonate
Small flashlight
Matches
Nickel for phoning for help

The aromatic spirit of ammonia can be used as a stimulant, by inhalation or internally by using a paper cup; also as a local antiseptic; as an antacid either locally or internally; and when ignited

for flaming a needle or blade. It is well to have on one's person a clean handkerchief and a scarf that can be used as a triangular bandage. A tongue depressor is handy for use in finger injuries. A bandage compress comes in handy in applying a dressing to oneself. Sodium bicarbonate can be used for dermatitis and also in eye cases as a wash. There should be available a small container of pure water.

The physician should carry in addition a hypodermic set with tablets of atropine sulfate, camphor, strychnine, morphine, caffeine sodium benzoate, Adrenalin, and also pearls of amyl nitrite. His bag should contain thermometer, stethoscope, sphygmomanometer, ephedrine, Adrenalin, 5 gr. quinidine sulfate tablets for fibrillation, 1/320 gr. ergotrate tablets for bleeding (and migraine), splints, roller bandages, sterile gauze and cotton, 1½ gr. aminophylline tablets for asthma, adhesive tape, tongue depressors, umbilical cord tape, instru-



Emergency Equipment for First Aid

ment case with haemostats, etc., sterile packing, mouth-gag, ether, tourniquet, suction apparatus for bites, antivenin, sterile outfit for culture, test tube and alcohol lamp for examining urine, sugar-test, osbtetrical forceps and an intubation set. A small generator light operated by hand is now on the market, which eliminates the possibility of battery failure in the conventional flashlight.

Automobile and auto-ambulance should carry oxygen in some form, Thomas splints, folding litter such as could be slid down a flight of stairs, blankets, pulmotor, long splints, and sterile towels. There is manufactured now a folding wheel-chair which may be useful in the transportation of the victim. In addition, it is well to have antidotes for the various poisons, a stomach-pump and an ice-cap.

The accident ward of a hospital is equipped with the usual solutions, etc., such as named above, tincture of green soap, sterile water, ethyl chloride, novocain, sterile dressings, litter, wheel-chairs, cots and blankets, alcohol sponges, mercury bichloride solution, olive oil, sterile syringes, stomach and rectal tubes, bandage scissors, adhesive rolls, sterilized instruments, autoclave and sterilizer, tincture of benzoin, "Ace" bandages, plaster of Paris, intravenous set, boric acid, Ichthyol and petrolatum, sodium chloride tablets, tincture of iodine, aromatic spirit of ammonia, sodium bicarbonate, bland ointments, iodoform gauze packing, Esmarch bandage and tourniquet, ice-caps and hot-water bags, barbiturates, hypodermic syringes, oxygen tanks and respirator, good drinking water and cups, table and chairs. The usual medicine cabinet, such as found in the home, contains aspirin, Brown mixture, Epsom salt, soda mint tablets, calomel, boric acid, castor oil, starch, "Band-aids," baking soda, bismuth, scissors, Ichthyol ointment, soap liniment, vinegar, alcohol, and dressings. The hospital has also insulin, penicillin, sulfonamides, tracheotomy set, etc. available.

With such provision, practically any accident case can be handled in the proper manner. Of course all medicines should be kept in bottles easily recognized and labelled, and also out of reach of children. Instruments should be neatly arranged and the room kept in order.

When one goes on a journey, surely a First Aid kit freshly prepared should be taken along and some calamine lotion, if there is probability of ivy poisoning. Antivenin can be hired for the hiking-trip. It is well to have on hand a suction pump (such as the ordinary breast pump). Have several triangular bandages, waterproof covering and material for building a fire along. Thus, many serious conditions may be prevented from occurring or promptly cared for should they occur.

SELECTED ABSTRACTS

Chronic Myelogenous Leukemia: A Study of 129 Cases in Which Treatment Was With Radioactive Phosphorus. J. W. Lawrence, R. L. Dobson, B. V. A. Low-Beer and B. R. Brown. *J. A. M. A. 136, 672* (1948). The authors report the results of the treatment of a series of 129 cases of chronic myelogenous leukemia with radiophosphorus alone or in conjunction with roentgen rays.

The P^{32} used in the more recent part of the study was derived from the S^{32} (n,p) P^{32} reaction which occurs with high yield when ordinary sufur is subjected to neutron bombardment in the atomic pile. The P^{32} was then converted into sodium monohydrogen phosphate, and this salt was used in a solution containing 15 to 18 mg. per cc. at a pH of approximately 7.4. During the last year of the investigation the solution was brought to isotonicity by the addition of sodium chloride. The beta ray activity of the solution was standardized against a uranium beta ray source in terms of microcurie radium equivalents. The solution was sterilized prior to administration by the oral or intravenous route.

The dosage was usually 1 to 2 millicuries once or twice a week, although doses equivalent to 40 millicuries intravenously over a period of seventy-two days were given in some instances. About one-half of the patients had received previous courses of roentgen therapy prior to the administration of radiophosphorus. Roentgen irradiation was given to about one-tenth of the remaining cases after initial P^{32} therapy had failed to produce the desired response.

The level and trend of the blood cell counts and the general clinical picture were the criteria used in the administration of radiophosphorus. In one-third of the cases acute leukemia developed terminally. It is believed that P^{32} exhibits a limited selective irradiation toward leukemic cells, but in this series no marked increase was noted in the duration of life of the majority of the patients. The fact, however, that 33 patients in this series have lived or are living five or more years after the onset of their symptoms is an indication that P^{32} may be the best agent available at the present for the therapy of chronic myelogenous leukemia.

Hydrogen Ion Concentration of Lachrymal Fluid. K. Pedersen-Bjergaard. *Pharm. J. 106,* 133 (1948). The author determined the average pH value of the lachrymal fluid of ten normal persons to be 8.2. Samples of 0.2-0.3 ml. were collected from each subject and the pH was determined immediately. The values observed for the samples collected from five males (ages 30 to 47) varied from 8.14 to 8.45 when determined electrometrically with the glass electrode, and from 8.0 to 8.4 colorimetrically. The pH values found for the samples obtained from five females (ages 23 to 57) were 8.02 to 8.41 (electrometrically) and 8.0 to 8.3 (colorimetrically).

Reference is made to several observers who have reported the pH value of lachrymal fluid to be of the same level as that of blood, viz., 7.25 to 7.40. A few authors have reported higher values, the highest being 8.4 to 8.6.

The author states that the osmotic concentration of lachrymal fluid corresponds exactly to that of blood.

It is claimed that the eye can well tolerate a 4.8 per cent aqueous solution of sodium sulfathiazole sesquihydrate; this solution is stated to be isontonic with lachrymal fluid, and to have a pH of 9.1.

A New Penicillin for Prolonged Blood Levels. N. P. Sullivan, A. T. Symmes, H. C. Miller and H. W. Rhodehamel, Jr. *Science 107,* 169 (1948). The use of wax in the Romansky formula for the prolongation of therapeutic blood levels of penicillin is stated to be the apparent cause for a number of unfavorable local reactions which have been noted. The authors report the observations made on a series of 21 patients who received procaine penicillin in cottonseed oil.

This drug is a crystalline penicillin complex prepared by chemically combining procaine with penicillin G. It is sparingly soluble, and may be administered in oil, oil and water emulsion, or physiological saline solution. Its solubility in these vehicles is stated to be approximately 7,000 units/cc. Procaine penicillin contains not less than 90 per cent penicillin G, with a potency of 940 units/mg. (1,040 units/mg. theoretical).

Other investigators have demonstrated the importance of particle size for the slow absorption of penicillin. In the work reported in this paper, at least 50 per cent of the total weight of the particles suspended in oil were 50μ or more in length.

Seventeen of the 21 patients received a dose of 1 cc., containing 300,000 units; the remainder received amounts of 2, 3 or 4 cc. Blood level determinations by Kolmer's method showed that at the 24th hour all patients except one had values of 0.062 or higher. Over one-third showed levels of 0.124. One patient who received 3 cc. (900,000 units) had a therapeutic blood level (0.031) at the 36th hour, and another who received 4 cc. (1,200,000 units) showed a blood level of 0.124 at the 48th hour. No local or systemic reactions were observed.

Levo-Arterenol. M. L. Tainter, B. F. Tullar and F. P. Luduena. *Science 107,* 39 (1948). The successful preparation of *l*-arterenol is reported, and a brief description of its physical and physiological properties is presented.

For a number of years it has been conjectured that 3,4-dihydroxyphenylethanolamine (arterenol, norepinephrine, noradrenalin) may be the sympathetic effector substance (sympathin E) rather than epinephrine, although only indirect evidence has been secured to support this hypothesis.

Numerous investigators have been unsuccessful in their attempts to resolve racemic arterenol into its optically active components. Tullar accomplished this by reacting equimolar quantities of racemic arterenol and *d*-tartaric acid in aqueous solution, from which the monohydrate of *l*-arterenol *d*-bitartrate crystallized. From the mother liquor crude *d*-arterenol *d*-bitartrate was obtained; this was purified by recrystallization from 90 per cent aqueous methanol. The respective optically active isomers of arterenol were prepared by treating aqueous solutions of these salts with ammonium hydroxide.

l-Arterenol occurs as a white crystalline substance which is freely soluble in water. Its solutions undergo slow oxidation under the influence of light and oxygen. The corrected melting points and optical rotations of the isomeric arterenols and of their hydrochlorides are reported.

l-Arterenol displayed more activity in raising the blood pressure of dogs than the *dl*- or *d*-forms. The *d*-form had only 3-4 per cent of the pressor activity of the *l*-, a ratio comparable to that observed in the epinephrine isomers. The activity of *l*-arterenol was 164± 10 per cent of that of *l*-epinephrine in dogs under phenobarbital anesthesia, giving to *l*-epinephrine a dosage ratio of 0.61 for equal effect, a figure agreeing with that of 1.2 previously reported for the racemic mixture. Cocaine sensitized the pressor response to *l*-arterenol to a greater degree than to *l*-epinephrine.

The acute toxicity of *l*-arterenol, the *d*-form, and *l*-epinephrine when administered by rapid intravenous injection was studied on mice, indicating that for equivalent pressor doses *l*-arterenol has a safety ratio (toxicity to pressor activity) approximately four times that of *l*-epinephrine.

The effect of *l*-arterenol on excised strips of rabbit and guinea pig ileum and on the nonpregnant rat uterus was also studied.

Toxicity of γ-Benzene Hexachloride in Clothing. R. G. Horton, L. Karel and L. E. Chadwick. *Science 107,* 246 (1948). Unpublished observations by Cross and Snyder indicate that herringbone twill impregnated with a standard test concentration of 2.0 gm. of the γ-isomer of benzene hexachloride (also known as hexachlorocyclohexane, GBH or "Gammexane") per square foot of cloth is highly effective against mites and withstands repeated launderings better than other proposed miticide impregnates.

The development of unusual symptoms in two workers who prepared batches of the compound for field tests suggested that the absorption of GBH through the skin was the causative factor. Tests were therefore performed on clipped laboratory animals; in one series, the toxicity of 40 per cent acetone solutions (*a*) of the pure γ-isomer or of (*b*) a mixture of hexachlorocyclohexanes containing 83 per cent of the γ-isomer was studied by direct application to the backs and sides. Precautions to avoid the ingestion or inhalation of the compound by the experimental animals were observed.

In another series, bleached herringbone twill cloths were impregnated with an acetone solution of a mixture of hexachlorocyclohexanes (83 per cent γ-isomer) in such manner as to yield a concentration

of 2 gm. of GBH/sq. ft. of cloth. The cloth, after air-drying, was applied snugly to the clipped trunks of the test animals. The covered area per unit body weight corresponded approximately to that for a man wearing a suit.

In decreasing order of sensitivity to GBH, the test animals were rabbits, rats and guinea pigs. The toxic symptoms noted were those characteristic for parenterally administered GBH: weakness, occasional flaccid paralysis, and finally periodic convulsions, generally leading to eventual death. The observation period was one week or longer. It was noted that bodily motion appeared to increase the rate of absorption of GBH in the clothed animals. Data collected in the two series of experiments are presented in tabular form.

The authors conclude that GBH appears to be sufficiently toxic to some mammals to warrant caution in its use as a miticidal impregnate at a concentration 2 gm./sq. ft. of clothing for man.

BOOK REVIEW

A Catalogue of Insecticides, Vol. I, Chemical Insecticides. By Donald E. H. Frear, Professor of Agricultural and Biological Chemistry, The Pennsylvania State College. Chronica Botanica Co., Waltham, Mass. (Stechert-Hafner, Inc., New York, N. Y.) 1947. xii+203 pp. 20 x 37 cm. Price $6.50.

This catalogue is the first of two volumes; the second to list the chemical fungicides. The work is a portion of Project 999, a World War II study made at the Pennsylvania Agricultural Experiment Station and is believed to be complete to January, 1944.

Each chemical compound has been coded, from code numbers assigned to each functional group, heterocyclic system, type of ring, open chain structure, unsaturation and also the cations and anions. Polyfunctional compounds are assigned code numbers, assigned in numerical order, for each of the structures present. The compounds are then tabulated according to increasing code number. Correct chemical names have been assigned to each compound. Listed also are the structural formulas, insecticidal uses, relative toxicities and literature references. A miscellaneous table lists a group of insecticides of questionable structure and those to which it is impossible to assign an empirical formula. Finally there is a patent list arranged according to country and number. It would have been well to include also a list of trade names of commercial insecticides.

The catalogue, which lists over 10,000 insecticidal agents, should be extremely valuable to anyone interested in this field. It certainly will save many hours of literature search.

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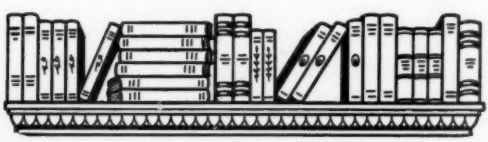

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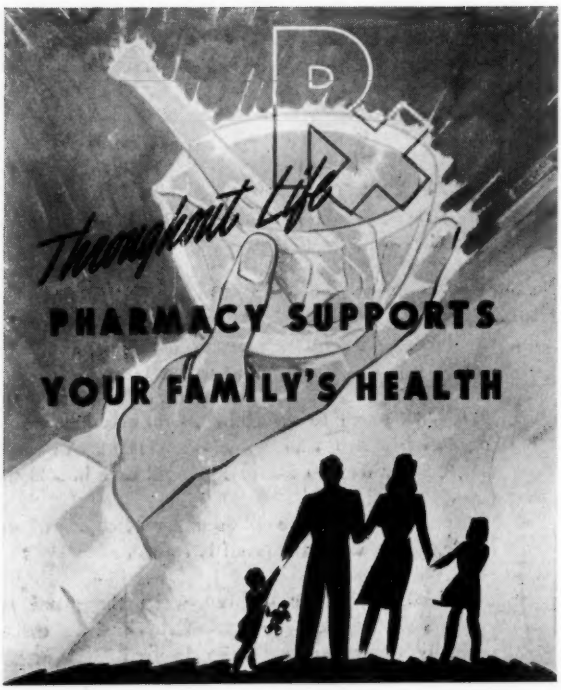
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